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To: C. K. Ellis **Date:** September 24, 1990

From: D. J. Ayers

Subject: Summary Planning Meeting Presentation - Biochemical Approach to the Reduction of TSNA

The biochemical approach to the reduction of mainstream TSNAs involves the reduction of preformed TSNA in filler. The more immediate goal of this project is to prevent nicotine accumulation in leaves by selectively eliminating an enzyme necessary for nicotine synthesis in root. The enzyme that catalyses the first step in the conversion of putrescine to nicotine, putrescine N-methyl transferase (PMT), is reported to be the most important enzyme in the control of nicotine production and therefore was selected as the first enzyme to investigate. Hydroponically grown tobacco plants are topped and the roots harvested three days after topping. The proteins are extracted from the roots via ammonium sulfate precipitation. The ammonium sulfate fraction is then applied to a phenyl-Sepharose column and the partially purified enzyme is eluted. The fraction of eluent from the phenyl-Sepharose column that has PMT activity is then exposed to DEAE "pseudoaffinity" chromatography. The PMT enriched sample that is eluted from the column is then concentrated and exposed to isoelectric focusing. Using this procedure, a sample was generated and submitted for protein sequencing. The first attempt to sequence this protein was successful in yielding a sequence of the first several amino acids. Plans include the confirmation of the amino acid sequence as that of PMT, determining more of the amino acid sequence, initiating work directed towards the isolation of the gene coding for PMT, transforming plants with an anti-sense construct of the gene, and ultimately measuring PMT and nicotine levels in the transformed plant.

cc: R. Kinser
Central Files

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